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OVERVIEW OF PRESENTATION

I. Background & Qualifications
II. Epidemiology Tutorial
III. Opinions and Work Performed
IV. Basis for Opinions
    A. Methodology
    B. Information Considered
    C. Bradford Hill
V. Conclusions & Ultimate Opinions

I. BACKGROUND & QUALIFICATIONS

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- Honors and Awards
- Representative Publications
- Editorial Work
- Research
- Noteworthy Projects and Grants
- Prior Expert Witness Work and Testimony

II. EPIDEMIOLOGY TUTORIAL

- Clinical Trials
- Observational Studies
- Meta-Analysis and Systematic Reviews
- Sources of Potential Error
  - Chance
  - Potential Bias
  - Confounding

CLINICAL TRIALS

Evidence from Randomized Clinical Trials would be ideal. "However, ***ethical and practical constraints*** limit the use of such experimental methodologies to assess the value of agents that are thought to be beneficial to human beings." *Ref. Guide on Epi.*; citing, *In re* Bextra & Celebrex Mktg. Sales Practices & Prod. Liab. Litig., 524 F. Supp. 2d 1166, 1181 (N.D. Cal. 2007).

OBSERVATIONAL STUDY DESIGNS

"Observational designs and methods are important for evaluating the safety of medicines post-approval. **They *may be the only means to evaluate a medicine's association*** with rare events or ***long-latency outcomes***."

REVIEW ARTICLE

Is the Large Simple Trial Design Used for Comparative, Post-Approval Safety Research?

A Review of a Clinical Trials Registry and the Published Literature

Robert F. Reynolds,[1,2] *Jemma A. Lee*,[1] *Nicolle M. Gatto*[1,2] and *Sybil M. Eng*[3]

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Contents

Abstract

Post-approval, observational drug safety studies face well known difficulties in controlling for confounding, particularly confounding by indication for drug use. A study design that addresses confounding by indication is the large simple trial (LST). LSTs are characterized by large sample sizes, often in the thousands; broad entry criteria consistent with the approved medication label; randomization based on equipoise, i.e. neither physician nor patient believes that one treatment option is superior; minimal, streamlined data collection requirements; objectively-measured endpoints (e.g. death, hospitalization); and follow-up that minimizes interventions or interference with normal clinical practice. In theory then, the LST is a preferred study design for drug and vaccine safety research because it controls for biases inherent to

OBSERVATIONAL EPIDEMIOLOGY (COHORT STUDY DESIGN)

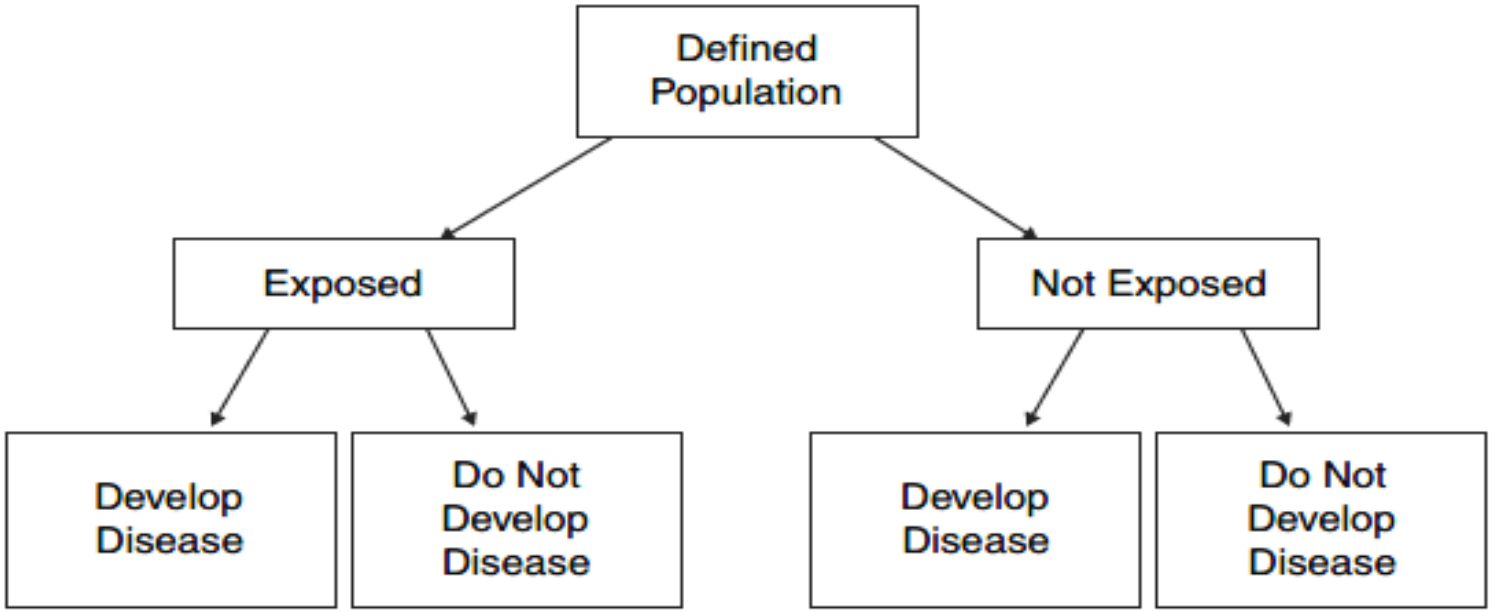
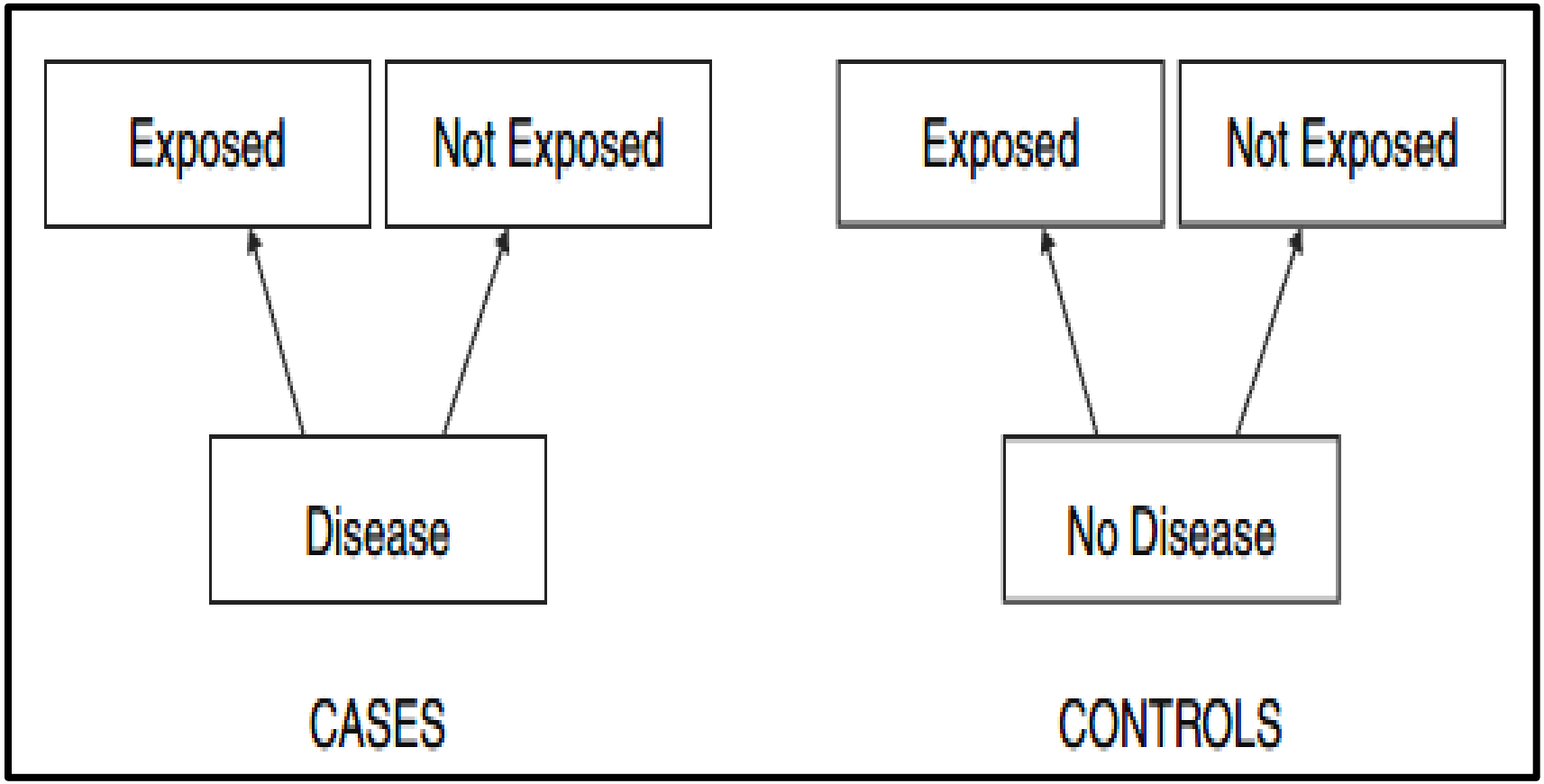


Table 1. Cross-Tabulation of Exposure by Disease Status

	No Disease	Disease	Totals	Incidence Rates of Disease
Not exposed	a	c	$a + c$	$c/(a + c)$
Exposed	b	d	$b + d$	$d/(b + d)$

OBSERVATIONAL EPIDEMIOLOGY (CASE-CONTROL STUDY DESIGN)



META-ANALYSIS

- Statistically combine study results from multiple studies;
- Provide greater statistical power than a single study
- Evaluation of statistical heterogeneity and consistency/inconsistency of results
- Evaluate risk of bias of individual studies
- Cannot overcome the individual biases of studies
- Needs to be preceded by a systematic review and ideally evaluate risk of bias of individual studies

BIAS

- Bias is a systematic error in the design, conduct, or analysis of the study.

RECALL BIAS

- Recall bias occurs when there are systematic differences in the way subjects remember or report exposures or outcomes.
- Usually more of a problem in case control studies
- Can result in estimates both away and towards the null
- Minimize by proper selection of controls, standardized questionnaires or validated markers

MISCLASSIFICATION OF EXPOSURE OR OUTCOME

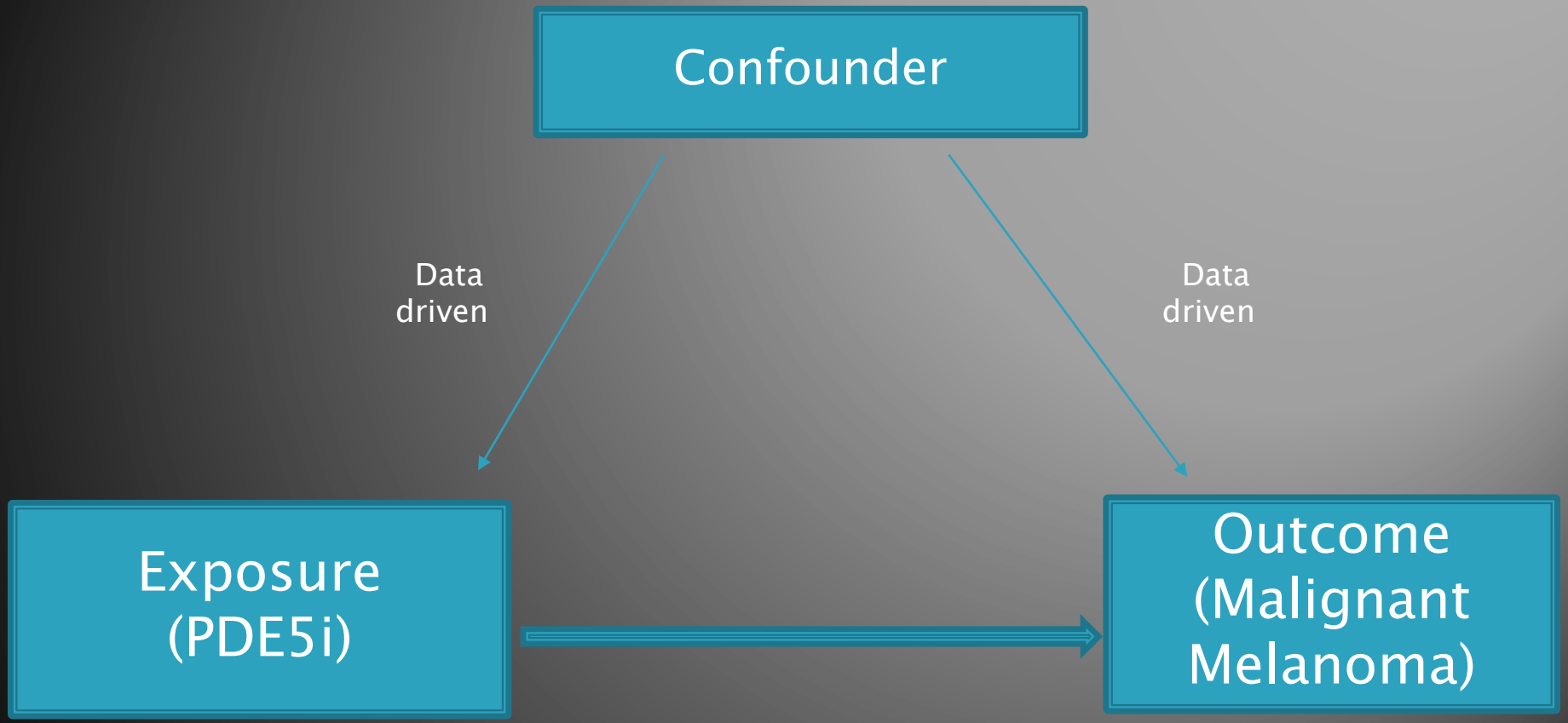
- Estimates of association can be biased if subjects are incorrectly categorized with respect to exposure status or outcome status.

- Non-differential misclassification of exposure or outcome usually biases towards the null.

CONFOUNDING VS RISK FACTORS

- Risk factors are those that are associated with the disease and not necessarily the exposure
- A confounder is a variable that is associated with the exposure and the outcome, but it is not a part of the causal pathway
- All risk factors ***are not*** confounders
- Need not adjust for all risk factors to generate unbiased estimates
- Over adjustment for risk factors/confounders may lead to attenuated estimates

CONFOUNDING



RELATIVE RISK

A commonly used approach for expressing the association between an agent and disease is relative risk ("RR"). It is defined as the ratio of the incidence rate (often referred to as incidence) of disease in exposed individuals to the incidence rate in unexposed individuals:

$$RR = \frac{\text{(Incidence rate in the exposed)}}{\text{(Incidence rate in the unexposed)}}$$

ODDS RATIO

	Cases (with disease)	Controls (no disease)
Exposed	*a*	*b*
Not exposed	*c*	*d*

In a case-control study,

$$\text{OR} = \frac{\text{(Odds that a case was exposed)}}{\text{(Odds that a control was exposed)}}.$$

The odds ratio represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

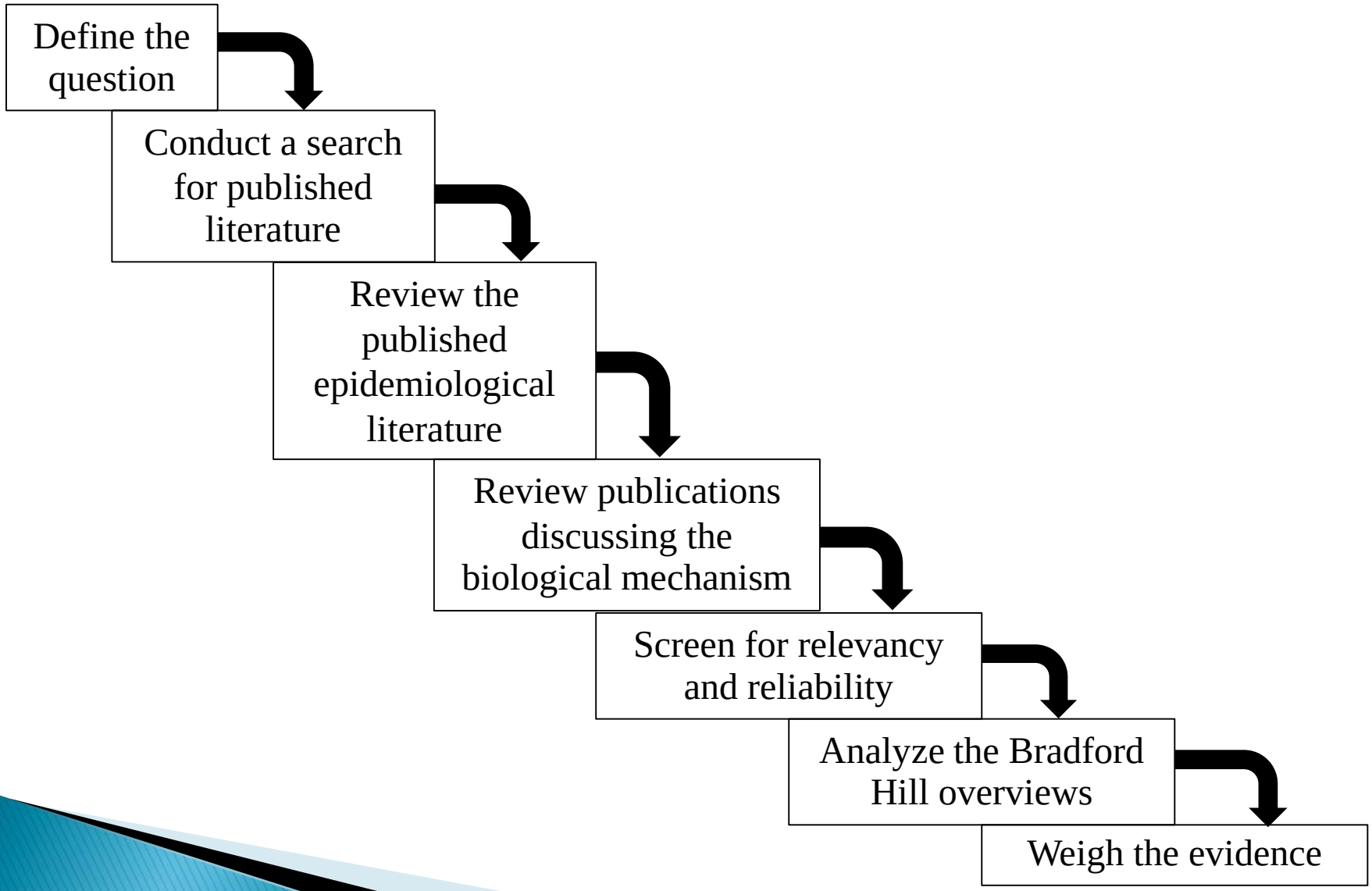
STATISTICAL SIGNIFICANCE

- A study that is *statistically significant* has results that are unlikely to be the result of random error or chance.
- HR 1.84 (95% CI, 1.04-3.22)
- Example with a p-value

III. OPINIONS & WORK PERFORMED

1. Objectives and Assignment
2. Summary of Work Performed
3. Information Considered (Totality of Relevant Scientific Evidence)
4. Methodology Overview
5. Summary of Ultimate Opinions

METHODOLOGY



MELANOMA EPIDEMIOLOGY

- Malignant melanoma is relatively rare, with an incidence rate of only 21.2 cases per 100,000 people.*
- Basal cell carcinoma is much more common, with an incidence rate of 226.1 cases per 100,000 people.**
- 91% of melanomas are diagnosed at an early stage (stage I or II).***
- Survival rate drops as melanoma spreads: 98.7% 5-year survival rate when confined to primary site versus 24.8% 5-year survival rate when spread to distant parts of the body****

*North American Association of Central Cancer Registries (NAACCR), 2018
**https://www.ncbi.nlm.nih.gov/pubmed?term=27473450
***Cancer Research UK
****https://seer.cancer.gov/statfacts/html/melan.html

LIMITS OF CLINICAL TRIALS

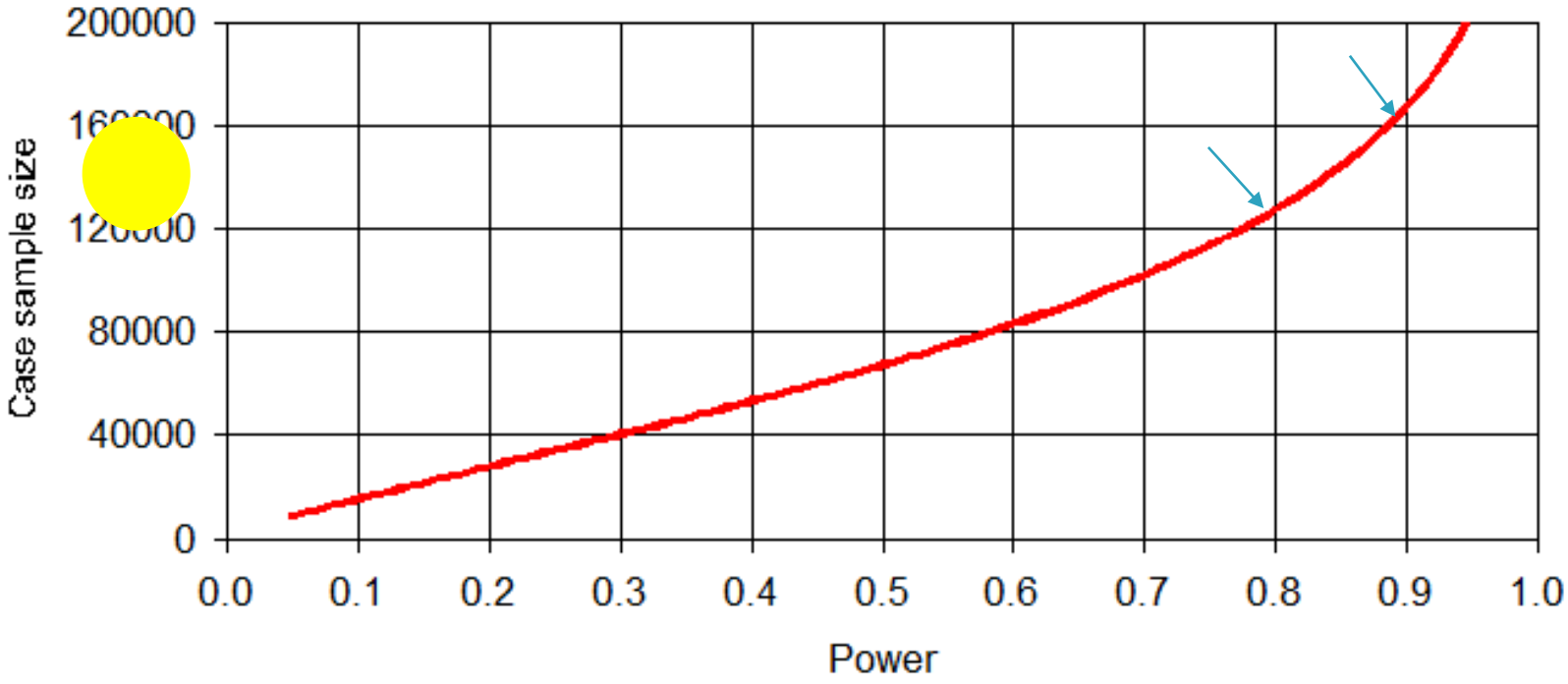
Detect Malignant Melanoma

- To detect a RR of 2
- Alpha of .05
- Power of 0.8
- Incidence Rate of MM = 21.2/100,000 #
- The sample size of the clinical trial would need to enroll 127, 523 participants in each arm using Fisher's exact test

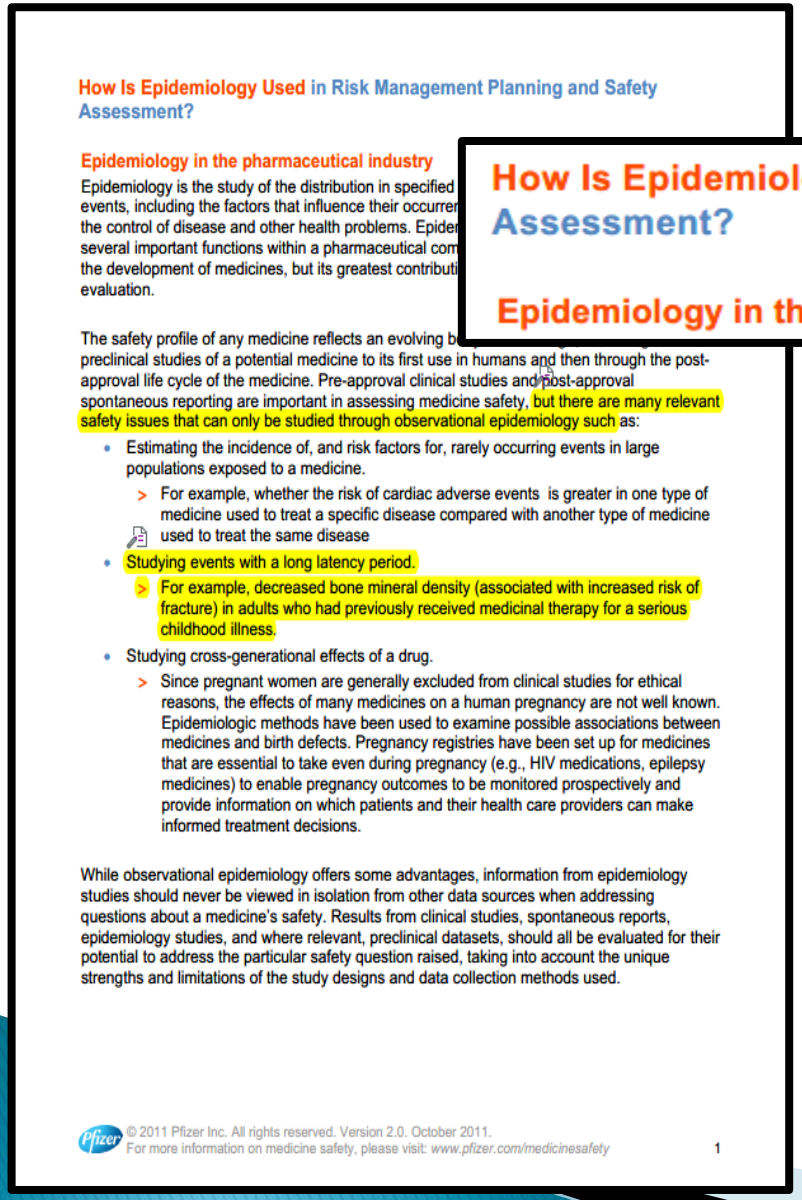
Data sources: North American Association of Central Cancer Registries (NAACCR), 2018
Dupont WD, Plummer WD: "Power and Sample Size Calculations: A Review and Computer Program", Controlled Clinical Trials 1990; 11:116-28.

LIMITS OF CLINICAL TRIALS

Sample-Size and Power



BEST EVIDENCE OF CAUSALITY



How Is Epidemiology Used in Risk Management Planning and Safety Assessment?

Epidemiology in the pharmaceutical industry

Epidemiology is the study of the distribution in specified
events, including the factors that influence their occurrer
the control of disease and other health problems. Epider
several important functions within a pharmaceutical com
the development of medicines, but its greatest contributi
evaluation.

The safety profile of any medicine reflects an evolving b
preclinical studies of a potential medicine to its first use in humans and then through the post-approval life cycle of the medicine. Pre-approval clinical studies and post-approval spontaneous reporting are important in assessing medicine safety, but there are many relevant safety issues that can only be studied through observational epidemiology such as:

- Estimating the incidence of, and risk factors for, rarely occurring events in large populations exposed to a medicine.
  - For example, whether the risk of cardiac adverse events is greater in one type of medicine used to treat a specific disease compared with another type of medicine used to treat the same disease
- Studying events with a long latency period.
  - For example, decreased bone mineral density (associated with increased risk of fracture) in adults who had previously received medicinal therapy for a serious childhood illness.
- Studying cross-generational effects of a drug.
  - Since pregnant women are generally excluded from clinical studies for ethical reasons, the effects of many medicines on a human pregnancy are not well known. Epidemiologic methods have been used to examine possible associations between medicines and birth defects. Pregnancy registries have been set up for medicines that are essential to take even during pregnancy (e.g., HIV medications, epilepsy medicines) to enable pregnancy outcomes to be monitored prospectively and provide information on which patients and their health care providers can make informed treatment decisions.

While observational epidemiology offers some advantages, information from epidemiology studies should never be viewed in isolation from other data sources when addressing questions about a medicine's safety. Results from clinical studies, spontaneous reports, epidemiology studies, and where relevant, preclinical datasets, should all be evaluated for their potential to address the particular safety question raised, taking into account the unique strengths and limitations of the study designs and data collection methods used.

For more information on medicine safety, please visit: www.pfizer.com/medicinesafety

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How Is Epidemiology Used in Risk Management Planning and Safety Assessment?

Epidemiology in the pharmaceutical industry

"There are many relevant safety issues that can only be studied through observational epidemiology such as: studying ***events with a long latency period***."

(PX173)

OBSERVATIONAL STUDIES DEMONSTRATE INCREASED RISK

Ten publications (11 total analyses) have attempted to address the risk of melanoma associated with PDE5 inhibitors

- Li et al, JAMA Intern. Med. (2014) – Prospective Cohort
- Loeb et al, JAMA (2015) – Nested Case Control
- Lian et al, European Ass'n of Urology (2016) – Prospective Cohort
- Matthews et al, PLOS Med. (2016) – Matched Cohort
- Pottegård et al, Br. J. Cancer (2016) – Matched Case Control (UK)
- Pottegård et al, Br. J. Cancer (2016) – Matched Case Control (USA)
- Boor et al (2016)* – Retrospective Cohort Study
- Ma et al, Mayo Clinic (2017) – Case Control
- Nardone et al (2018)* – Retrospective Analysis of AE's (RADAR project)
- Shkolyar et al, J. Sex. Med. (2018) – Retrospective Cohort
- Christie et al, J. Urology (2019) – Case Control

- Analysis of AE's from RADAR project database

(JX90, JX93, JX91, JX94, JX96, JX101, JX109, JX113, JX97, & PX72)

LI, ET AL., 2014

- Prospective cohort design to assess temporality
- Primary analysis showed an 84% increased risk for recent users of sildenafil (95% CI, 1.04-3.22)
- Multivariate adjustment for major confounders such as sun exposure and many others
- Health professionals with relatively homogenous SES
- Validation of melanoma outcome in majority of cases

LOEB, ET AL., 2015

- Nationwide, population-based, nested case-control study in the Swedish Prescribed Drug Register, the Swedish Melanoma Register, and other registers and databases in Sweden.
- Primary analysis showed a statistically significant 21% increased risk of melanoma for men taking PDE5 inhibitors (95% CI, 1.08-1.36).
- PDE5 inhibitors were significantly associated with stage 0 melanoma (OR, 1.49 [95% CI, 1.22-1.83]; 13% for cases vs 8% for controls).
- Risk estimates were similar for sildenafil, vardenafil or tadalafil.

LIAN, ET AL., 2016

- A cohort study of men newly diagnosed with ED between 1998 and 2014 using the UK Clinical Practice Research Datalink
- Results of the primary analysis showed an 18% increased risk for PDE5 inhibitor users versus non-users (95% CI, 0.95-1.47).
- However increased risk with increasing number of prescription - 7 prescription resulted in HR of 1.34

MATTHEWS, ET AL., 2016

- Embedded case control study using primary care data from the UK Clinical Practice Research Datalink
- The primary analysis found a 14% increased risk of melanoma for PDE5 inhibitor users that was statistically significant (95% CI, 1.01-1.29)
- Fairly consistent results showing increased risks above 1.0, although not all are statistically significant

POTTEGÅRD, ET AL, 2016

- Conducted two independent case-control studies using the Danish Nationwide Health Registries (DNHR) and using electronic health records at Kaiser Permanente Northern California (KPNC)

- Both studies found a 6% (DNHR) and a 1% (KPNC) increased risk of melanoma with ever use of PDE5 inhibitors but not statistically significant.

- Exposure was defined as two or more filled prescriptions of any PDE5 inhibitor, while non-use was defined as none or one filled prescription

SHKOLYAR, ET AL., 2018

- Retrospective cohort study of subjects contained within the Truven Health MarketScan claims database of insurance claims information in the United States from 2007-2015

- There was a 5% increased risk of melanoma for PDE5 inhibitor users that was statistically significant

CHRISTIE, ET AL., 2019

- Retrospective database review using the Veterans Affairs database for Veterans who received PDE5i treatment for ED compared to no-users (1.27 million in each group).

- The primary analysis reported a 25% increased risk that was statistically significant.

THREE ABSTRACTS

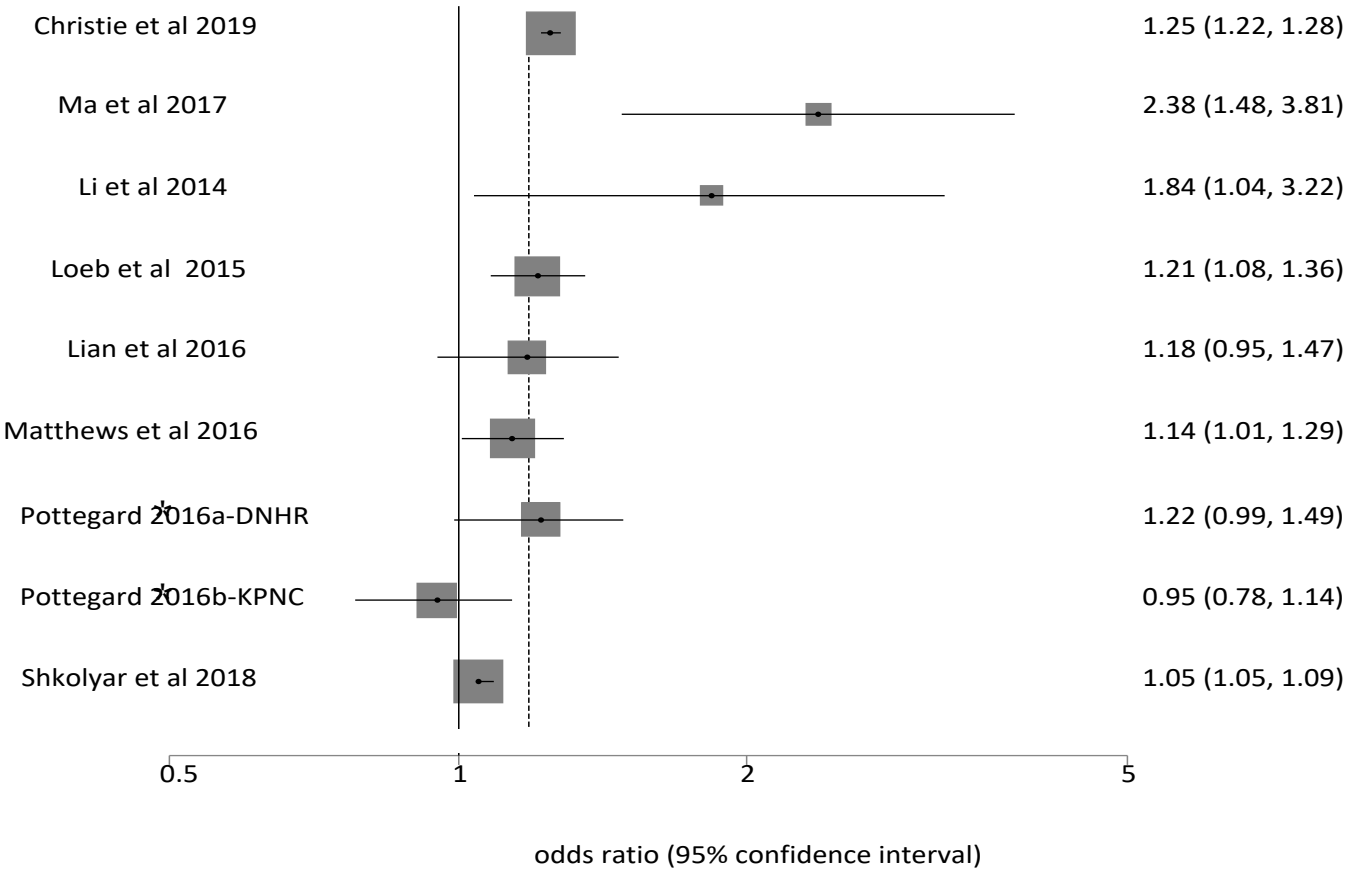
Ma et al, In both the univariate and multivariate logistic regression analyses, there was a significantly increased risk of malignant melanoma in men taking sildenafil (OR, 2.02 [95% CI, 1.32-3.09]; OR, 2.38 [95% CI, 1.49-3.81]). Sildenafil users were associated with developing additional primary melanoma (17.6%) compared to non-users (8.8%), P .077."

Boor et al, Results: After adjustment for race and age, a significant association with melanoma was detected for T (OR:1.6; 1.11-2.31, P =0.01) and for S (OR: 2.07; 1.48-2.89, P<0.001).

Nardone et al: "After adjusting for age, gender, and race, [the study found] a significant association for Malignant Melanoma" for both Sildenafil and Tadalafil. OR 2.98 (CI: 1.15-7.75, p=0.02)"
"results from this study may also ***suggest a dose-related effect*** for risk of subsequent MM …."

(JX109, JX101, & JX113)

OBSERVATIONAL STUDIES ARE CONSISTENT



*These estimates reflect the high use data included in the Pottegard abstract. The results for ever use were 1.06 (0.96-1.18) (DNHR) and 1.01 (0.91-1.12) (KPNC)

(PX72, JX109, JX90, JX93, JX91, JX94, JX96, & JX97)

META-ANALYSIS DEMONSTRATE INCREASED RISK

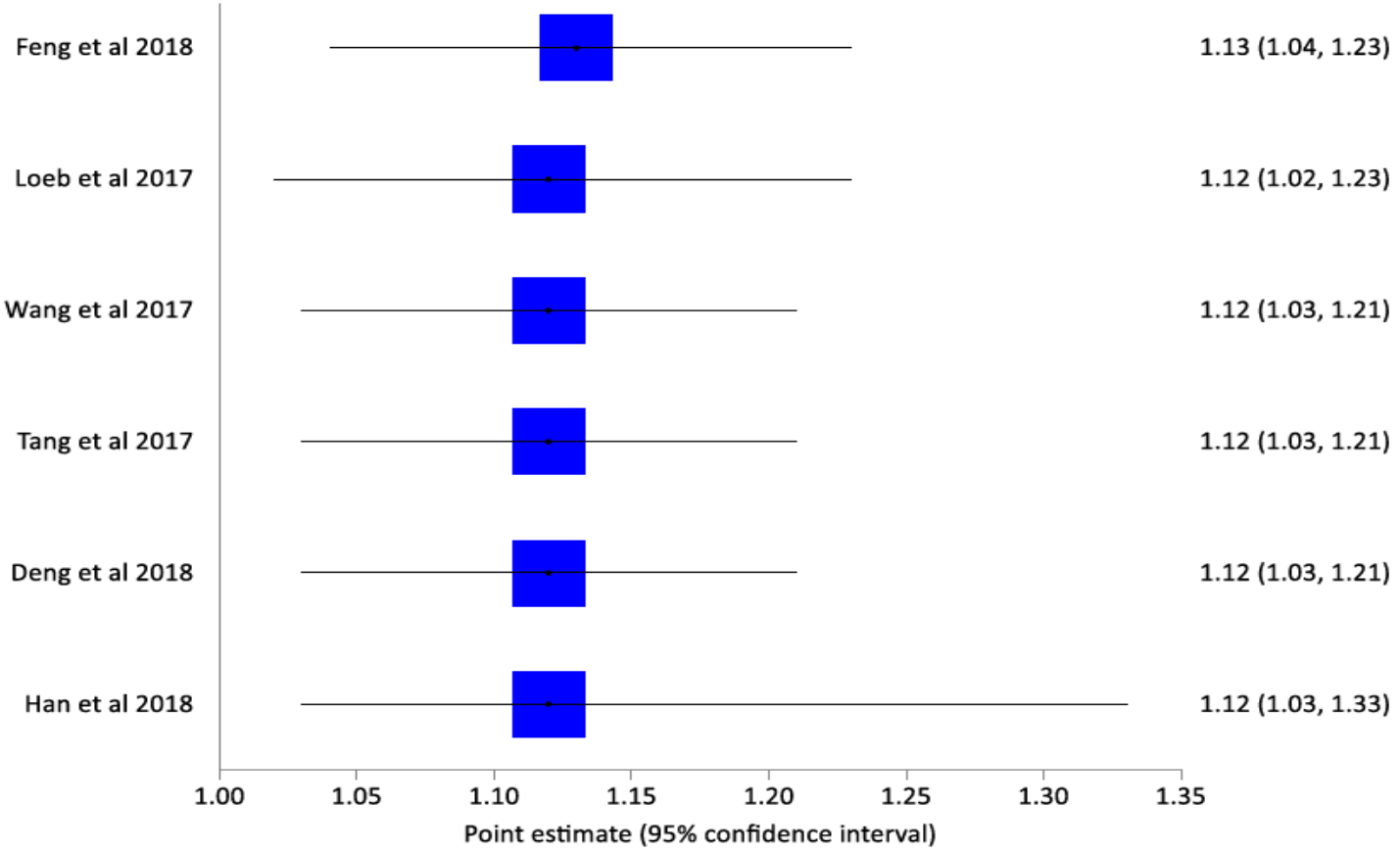
To date there are six (6) meta-analyses evaluating the association between PDE5Is and malignant melanoma. Although some of them have different inclusion criteria for studies, the consensus is that PDE5i use is associated with a significantly increased risk for melanoma.

- Wang J, Shen Y, Wang J, et al. Relation of phosphodiesterase type 5 inhibitors and malignant melanoma: a meta-analysis and systematic review. Oncotarget. 2017;8:46461–46467.
- Loeb S, Ventimiglia E, Salonia A, et al. Meta-analysis of the association between phosphodiesterase inhibitors (PDE5Is) and risk of melanoma. J Natl Cancer Inst. 2017;109.
- Deng T, Duan X, Liu B, et al. Association between phosphodiesterase type 5 inhibitors use and risk of melanoma: a meta-analysis. Neoplasma. 2018;65:216–221.
- Feng S, Zhou L, Liu Q, et al. Are phosphodiesterase type 5 inhibitors associated with increased risk of melanoma?: a systematic review and meta-analysis. Medicine (Baltimore). 2018;97:e9601.
- Han X, Han Y, Zheng Y, et al. Use of phosphodiesterase type 5 inhibitors and risk of melanoma: a meta-analysis of observational studies. Onco Targets Ther. 2018;11:711–720
- Tang H, Wu W, Fu S, et al. Phosphodiesterase Type 5 inhibitors and risk of melanoma: a meta-analysis. J Am Acad Dermatol.2017;77:480–488. https://doi.org/10.1016/j.jaad.2017.04

(JX99, JX92, JX86, JX88, JX89, & JX98)

Six independent meta-analysis show statistically significant excess risk

Overview of Metaanalysis of Phospodiesterase-5 inhibitors and malignant melanoma



(JX88, JX92, JX99, JX98, JX86, & JX89)

CLASS EFFECT

Appropriate to consider evidence from other PDE5 *specific* inhibitors

- Similar chemical structure
- Similar pharmacokinetic properties
- Similar mechanism of action
- Similar therapeutic effect
- Outcome measured
- Other independent experts looked at evidence from other PDE5 inhibitors when considering the risk of melanoma
- Both Pfizer and Lilly considered evidence from other PDE5 inhibitors when evaluating the risk of melanoma.

MECHANISM OF ACTION – AROZARENA 2011

- **Summary:**
  - PDE5 inhibitors promote cell invasion….
  - Through PDE5A inhibition, the drugs mimic an affect of gene activation and may function as a trigger for the creation of melanoma cells
  - "PDE5A drugs could promote melanoma metastasis" because "melanoma cells can rapidly evolve to become invasive, so any acceleration of this process is undesirable."

Cancer Cell

Article

Oncogenic BRAF Induces Melanoma Cell Invasion by Downregulating the cGMP-Specific Phosphodiesterase PDE5A

Imanol Arozarena,[1,3] Berta Sanchez-Laorden,[1] Leisl Packer,[1] Cristina Hidalgo-Carcedo,[2] Robert Hayward,[1] Amaya Viros,[1] Erik Sahai,[2] and Richard Marais[1,*]

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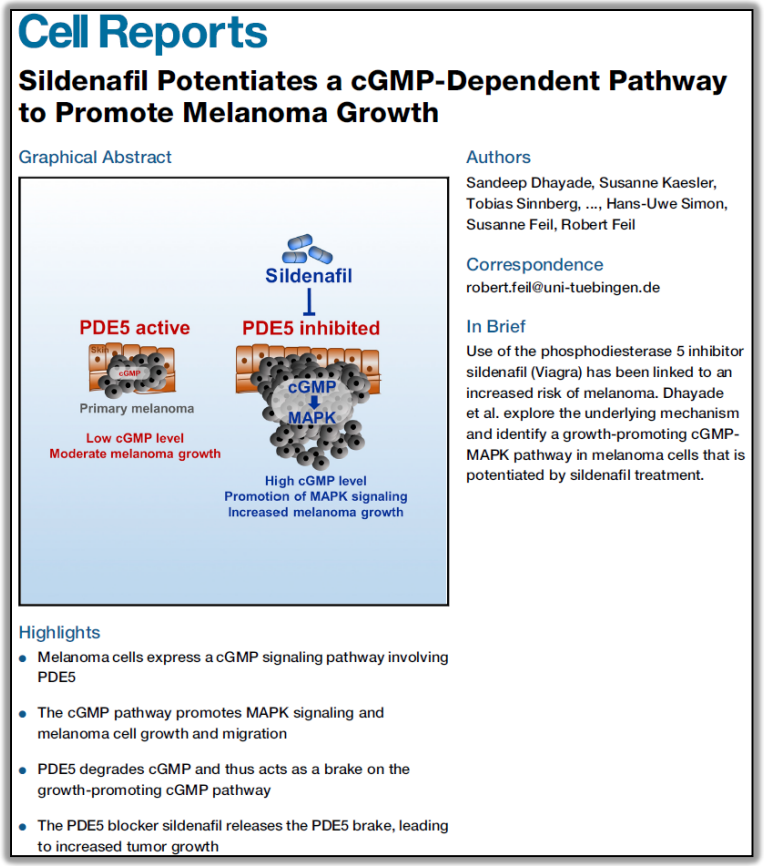
DOI 10.1016/j.ccr.2010.10.029

SUMMARY

We show that in melanoma cells oncogenic BRAF, acting through MEK and the transcription factor BRN2, downregulates the cGMP-specific phosphodiesterase PDE5A. Although PDE5A downregulation causes a small decrease in proliferation, its major impact is to stimulate a dramatic increase in melanoma cell invasion. This is because PDE5A downregulation leads to an increase in cGMP, which induces an increase in cytosolic Ca^{2+}, stimulating increased contractility and inducing invasion. PDE5A downregulation also this leads to an increase in short-term and long-term colonization of the lungs by melanoma cells. We do not observe this pathway in NRAS mutant melanoma or BRAF mutant colorectal cells. Thus, we show that in melanoma cells oncogenic BRAF induces invasion through downregulation of PDE5A.

MECHANISM OF ACTION – DHAYADE 2016

- **Summary:**
  - Melanoma cells express cGMP signaling pathway involving PDE5
  - The pathway promotes MAPK signaling and melanoma cell growth and migration
  - PDE5 degrades cGMP
  - Thus, PDE5 acts as a "brake" on the growth-promoting cGMP pathway
  - **Sildenafil, by inhibiting PDE5, releases the brake leading to increased tumor growth**



Cell Reports

Sildenafil Potentiates a cGMP-Dependent Pathway to Promote Melanoma Growth

Graphical Abstract

Highlights

- Melanoma cells express a cGMP signaling pathway involving PDE5
- The cGMP pathway promotes MAPK signaling and melanoma cell growth and migration
- PDE5 degrades cGMP and thus acts as a brake on the growth-promoting cGMP pathway
- The PDE5 blocker sildenafil releases the PDE5 brake, leading to increased tumor growth

Authors
Sandeep Dhayade, Susanne Kaesler, Tobias Sinnberg, ..., Hans-Uwe Simon, Susanne Feil, Robert Feil

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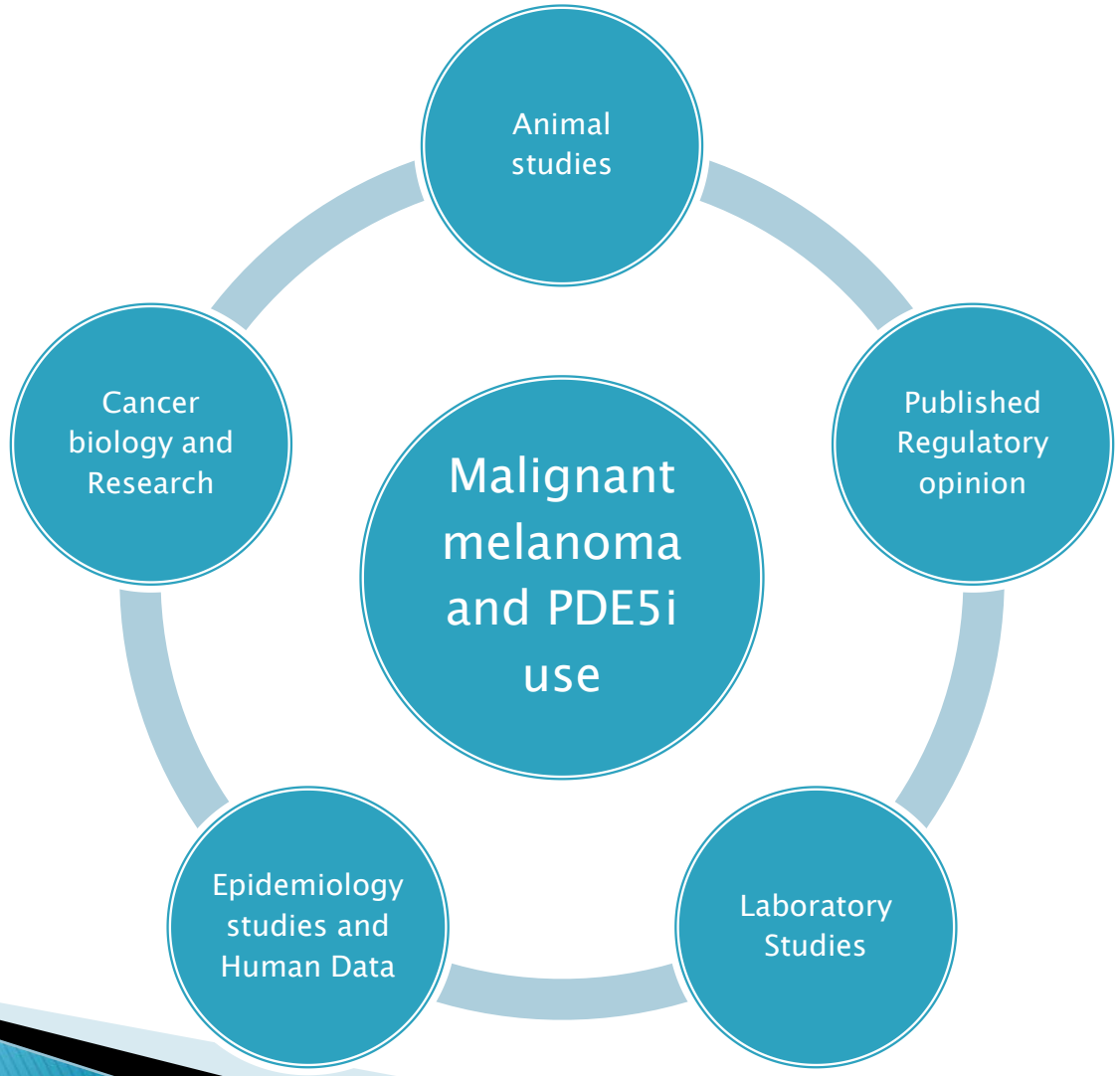
In Brief

Use of the phosphodiesterase 5 inhibitor sildenafil (Viagra) has been linked to an increased risk of melanoma. Dhayade et al. explore the underlying mechanism and identify a growth-promoting cGMP-MAPK pathway in melanoma cells that is potentiated by sildenafil treatment.

METHODOLOGY
TOTALITY OF EVIDENCE



BRADFORD HILL ANALYSIS

- ***Strength***
- ***Consistency***
- ***Specificity***
- ***Temporality***
- ***Biological gradient***
- ***Plausibility***
- ***Coherence***
- ***Experimental evidence***
- ***Analogy***

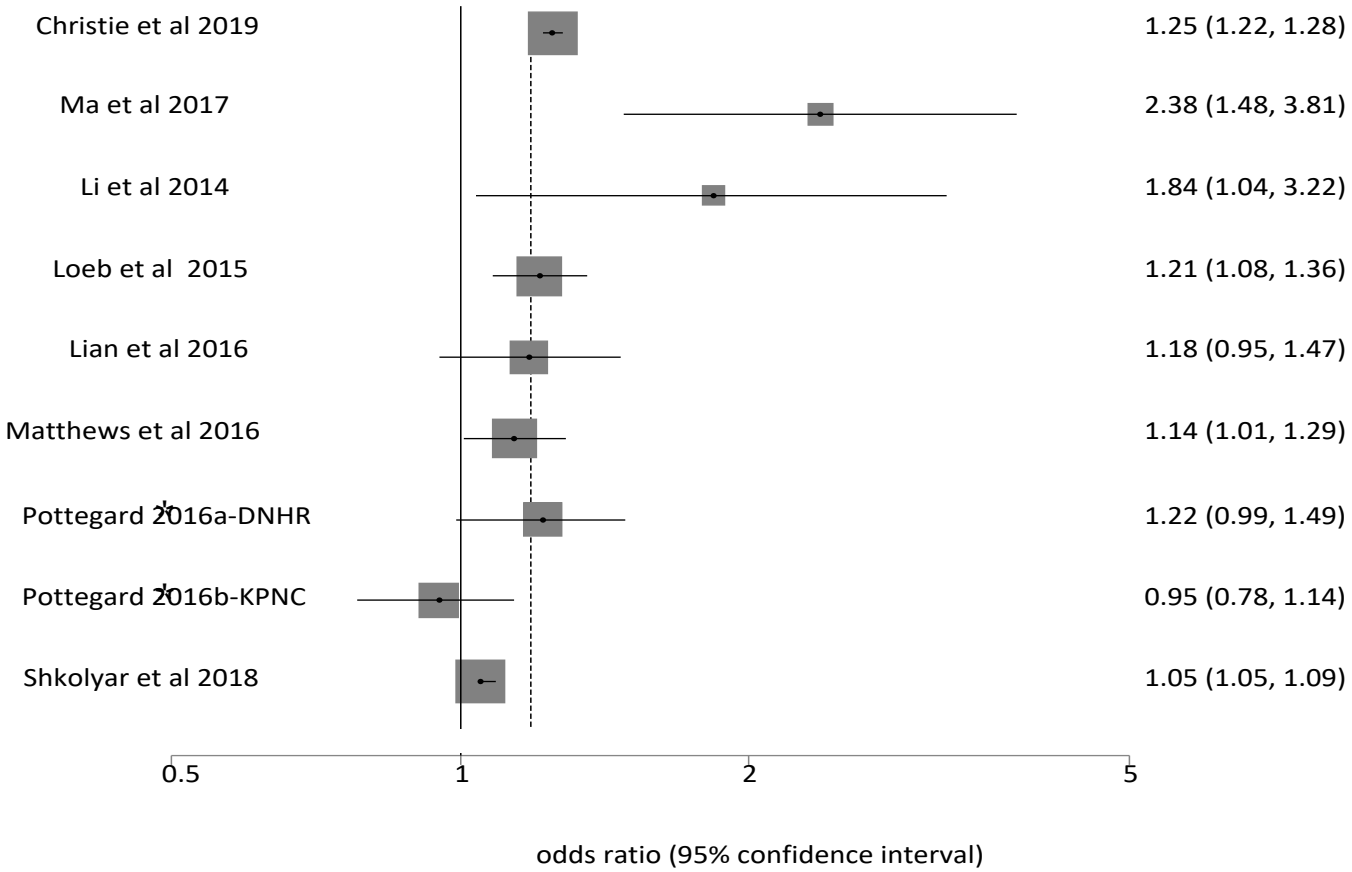
Source: Sir Austin Bradford Hill, The Environment and Disease: Association or Causation, *58 Proc. R. Soc. Med. 295*

BRADFORD HILL ANALYSIS (Strength of Association)

- The higher hazard ratios from Li are particularly significant given that it was the only study to control for sun exposure

- Almost every study and meta-analysis shows an increased risk and a large majority are statistically significant.

BRADFORD HILL ANALYSIS (Consistency)



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(PX72, JX109, JX90, JX93, JX91, JX94, JX96, & JX97)

BRADFORD HILL ANALYSIS (Specificity)

- The presence of this criterion is not really possible in most cancer cases.

- Even well-established causal relationship like cigarette smoking and lung cancer are not characterized by specificity.

BRADFORD HILL ANALYSIS (Temporality)

- Clearly established in the prospective cohort study by Li et al.

- Also demonstrated in the case-control studies which were embedded in cohorts by Lian et al. and Matthews et al.

(JX90, JX91 & JX94)

BRADFORD HILL ANALYSIS (Dose Response)

- There is not conclusive evidence of a linear, monotonic dose response, but there is some data that is suggestive where the higher dose levels have the higher risks.
- No study fully considers all 3 pieces of dose: dosage, duration, and frequency.
- Mechanistic studies in this case raise the possibility of a trigger effect, rather than a cumulative or linear dose response.

(JX91, JX96, & JX113)

BRADFORD HILL ANALYSIS (Biological Plausibility)

- A biological mechanism that links the exposure to the outcome that is consistent with current scientific knowledge
- Not the same as biological certainty
- Clearly demonstrated by mechanistic studies and acknowledged in multiple epidemiological studies

BRADFORD HILL ANALYSIS (Coherence)

- Minimal sacrifice of current scientific knowledge must be made for this association to be causal.

- The totality of the evidence easily fits within the current framework of scientific knowledge about the links between PDE5 inhibitor exposure and the development of malignant melanoma.

BRADFORD HILL ANALYSIS (Experiment)

- Practical issues
- It is unethical to conduct an RCT to prove melanoma risk.
- Admissions that none of the RCTs were ever sufficiently powered (both time and number of study participants)
- Pfizer admission that Observational Studies are best to determine risk for adverse events like melanoma.

BRADFORD HILL ANALYSIS (Analogy)

- Immunosuppresants, diuretics, and β-adrenergic blocking agents are all examples of drugs that are associated with increased risks of malignant melanoma.

- This demonstrates that there are drugs known to cause an increased risk of melanoma.

CONSIDERATION OF POTENTIAL ALTERNATIVE EXPLANATIONS

- Lifestyle Factors

CONSIDERATION OF POTENTIAL ALTERNATIVE EXPLANATIONS

- Sun Exposure

CONSIDERATION OF POTENTIAL ALTERNATIVE EXPLANATIONS

- Health-Seeking Behavior

WEIGHING THE EVIDENCE

- Qualitative judgment about causality
- Some factors are emphasized more than others based on the question at hand and the evidence available
- Not a simple checklist

CONCLUSIONS & OPINION

- Based on my review of the available evidence, it is my opinion that use of PDE5 inhibitors, like sildenafil, are capable of causing melanoma.
- A biologically plausible mechanism of action exists explaining how inhibition of PDE5 causes melanoma.
- My assessment of the Bradford Hill factors confirms this causal association.
- My opinions are expressed to a reasonable degree of medical and scientific certainty and based on my training, education, research and experience and are in accordance with the generally accepted standards of the scientific and epidemiological communities.